Characterizing the Hematologic and Plasma Chemistry Profiles of Captive Crested Geckos (*Rhacodactylus ciliatus*)

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Abstract: Blood samples were collected from 18 female and 20 male adult crested geckos from 1 captive population in Massachusetts, USA. The geckos were given a thorough examination, and a blood sample was collected for a complete blood count (CBC) and plasma biochemistry analysis. Whole blood was stored in lithium heparin for analysis. The median weight of the geckos was 43.4 g (10–90%: 38.0–55.3; min–max: 32.0–69.0). The range of the packed cell volume (PCV) for the geckos was 23.0–45.0%. The lymphocyte was the most common leukocyte found on the CBC. The following parameters were found to be significantly different by sex: calcium (P = 0.0001), phosphorus (P = 0.0001), total protein (P = 0.03), albumin (P = 0.03), and PCV (P = 0.001). Overall, the geckos in this study were clinically healthy, and the ranges established from this population may be used as a basis for comparison in clinical cases.

Key Words: Blood, crested gecko, hematology, plasma biochemistry, *Rhacodactylus ciliatus.*

Introduction

The crested gecko is a fairly recent addition to the ever-growing popular pet reptile market. The species was first described in 1866 as *Correlophus ciliatus*, but has since been renamed *Rhacodactylus ciliatus*. Until 1994 the species was believed to be extinct; however, at that time it was rediscovered in New Caledonia. Because of their ease of care, crested geckos are relatively easy to keep healthy in captivity (de Vosjoli *et al.*, 2003). We feel that this species will become even more popular in the future and insight to their clinical pathology is needed.

Like all *Rhacodactylus* geckos, crested geckos have webbing on the legs and digits. A vivarium for one of these geckos should be 1.2–1.8 m (4–6 feet) tall; however, these geckos also do well in small enclosures such as a 75 L (20-gallon) glass tank (24 in. × 12 in. × 16 in.; 61 cm × 30.5 cm × 40.6 cm). Just as with all tropical species, good ventilation is important. The temperature does not need to be significantly higher than standard room temperature (75°F; 25°C), and most people do not install separate heating elements for the vivarium. Misting the vivarium on a daily basis will help maintain a high relative humidity (60–70%). Most breeders separate the females from the males for a few of months out of the year. The geckos are primarily nocturnal, and in captivity will generally spend the daylight hours sleeping in a secure hiding place close to or near the substrate. In the wild, crested geckos rarely emerge from the canopy, because larger geckos living in the tree trunk and brush will readily eat them. Because of the cryptic behavior of these animals, an ultraviolet light source is not considered necessary, as captive animals have been found to thrive and breed successfully without any special lighting. However, one of the authors (JM) generally recommends a full-spectrum light source for at least 2–4 h a day, as this would mimic UV exposure of the natural environment and will most likely not cause any detrimental effects.

This species of gecko is a true omnivore, eating a variety of insects and fruit. Small insects should be fed in addition to fruit-based baby food or fruit mash, which can be easily supplemented with vitamins and minerals. There are also commercial diets on the market that can be used in lieu of baby food.

Currently, the export of wild New Caledonian crested geckos is prohibited. Fortunately, breeding in captivity is relatively easy, assuring a good supply of new animals for the pet trade without posing a threat to the wild populations. Longevity records for crested geckos are lacking, although they are thought to have the ability to live for up to 20 yr in captivity.

Materials and Methods

Crested geckos—This study was approved as a clinical research protocol (013-07B) by the review committee of the Cummings Veterinary School at Tufts University (North Grafton, MA). The population consisted of captive-bred crested geckos acquired from a reptile breeding facility in Massachusetts. The gender ratio of crested geckos in the study was 21:19 (male:female). Individual geckos originated from over 15 different reptile breeding facilities. Eight geckos in the study were produced at the Massachusetts breeding facility. The geckos ranged in age from 1 yr 7 months to 11 yr 10 months. Females ranged in age from 1 yr 7 months to 4 yr of age, and males ranged from 2 yr to 11 yr 10 months of age. The geckos ranged in weight from 32 to 60 g. The blood samples were collected within a period of 1 month. All females were housed separately from the males during the blood collection dates, and had been rested for at least 3 months prior to the initial blood collection date. Prior to the study, fecal samples from a representative sample of the colony were combined and analyzed with the use of a fecal smear and zinc sulfate flotation. No flagellates, protozoans, cestodes, trematodes, or nematodes were detected. Some of the geckos had been treated with metronidazole and/or fenbendazole approximately 3 yr before the study. The captive husbandry was the same for all of the crested geckos. Males were housed singly or with females, because males kept together may fight and injure each other. Females were kept with males, singly, or in groups of two to three females. Geckos were housed in terrariums of varying sizes. Some single geckos were kept in 45.7 cm × 30.5 cm × 50.8 cm (18 in. × 12 in. × 20 in.) fresh-air screen habitats, some pairs were kept in 61.0 cm × 30.5 cm × 40.6 cm (24 in. × 12 in. × 16 in.) glass tanks, and other pairs and trios were kept in 45.7 cm × 45.7 cm × 61.0 cm (18 in. × 18 in. × 24 in.) extra-large terrariums. All glass tanks had screen tops. For sanitary purposes and ease of cleaning, substrate, plants, food and water dishes, and climbing materials were disposable. Substrate consisted of folded paper towel. Recycled pulp paper egg trays (30.5 cm × 30.5 cm × 5.0 cm; 12 in. × 12 in. × 2 in.) were stacked creatively to provide more surface area for climbing and additional hiding spots. Artificial ivy was strewn about the terrarium and/or attached to the walls of the enclosures with a suction cup and hook. Paper towel rolls and toilet paper rolls were also provided for comfort and hiding. No heat source or special lighting was provided to any of the geckos, and room temperatures varied between 23.3 and 25.5°C (74 and 78°F). Two 60-watt light bulbs illuminated the room for approximately 14 h a day, after which they were turned off for the night. Cages were misted with a spray bottle daily to help adult geckos shed and to provide humidity. Because crested geckos climb on the walls of the enclosure, terrariums would be misted down with a spray bottle full of water and then wiped clean with paper towel.

The geckos had access to water at all times. Crested geckos received fruit-based baby food (peach, banana, and occasionally mango flavored) 4 days a week. Feeding portions were approximately the size of a quarter (~2.5 cm by 2 mm; 1.0 × 0.08 in.) per gecko. Chicken- or turkey-based baby food was mixed in with the fruit-based baby food once every 2 wk (one part meat:three parts fruit). Large crickets (19 mm; 3/4 in.) were fed to the geckos 2 days a week instead of baby food. Geckos were provided with calcium and vitamin supplementation for two feedings in a row, with a feeding day off in between. Equal parts of a phosphorus-free calcium supplement with vitamin D_3 and a multivitamin supplement with beta-carotene were added to the food. Supplements were provided as directed (1/2 tbsp of each supplement per pound of food) and mixed into the baby food. Crickets were also dusted with the same supplements in equal proportions.

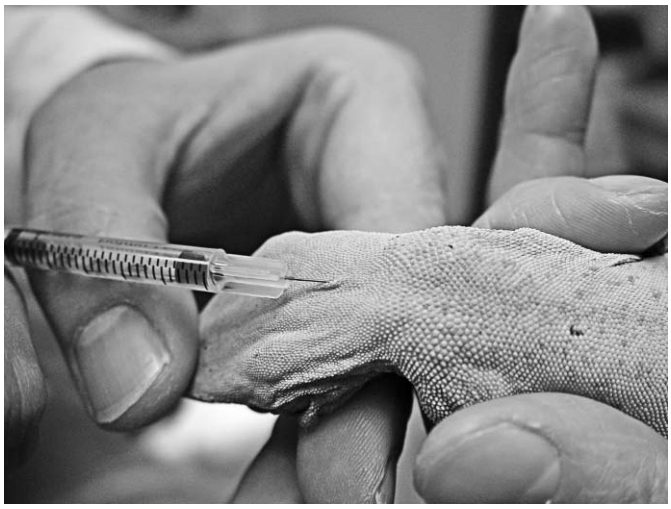

Figure 1. The venipuncture technique used to access the cranial vena cava in the crested gecko.

For venipuncture, the geckos were manually restrained and blood was collected from the cranial vena cava or by cardiocentesis with the use of an insulin syringe with a 27-gauge needle (Fig. 1). The physical exam and the phlebotomy was conducted by the same person for all animals. A maximum blood volume of 0.3 ml was taken. Blood was immediately transferred to a lithium heparin container, and the samples were processed immediately after collection.

Hematologic analysis—Red blood cell (RBC) mass was determined by measuring the packed cell volume (PCV) with the use of standard centrifugation of microhematocrit tubes. Heterophil/eosinophil counts were performed manually with the use of a hemocytometer and a Unopette designed for counting eosinophils (Becton Dickinson and Company, Franklin Lakes, NJ). Blood smears were air dried and stained with the use of an Aerospray 7120 automated hematology slide stainer (Wescor, Inc., Logan, UT) with an aqueous Romanowsky stain (Hemaspray, Thermo Fisher Scientific, Waltham, MA), and 200 cells were evaluated for the differential leukocyte counts. Hematologic analysis for all animals was conducted by the same person. Leukocytes were classified as heterophils, lymphocytes, monocytes, eosinophils, or basophils. The total white blood cell count (WBC) was calculated by correcting the manual count for the percentage of heterophils and eosinophils present (Campbell and Ellis, 2007). Thrombocyte numbers were estimated in two ways: (1) by determining the number of thrombocytes seen in each × 100 microscopic field, and (2) documenting the number of thrombocytes seen in the course of identifying 100 leukocytes.

Table 1. Crested gecko hematologic and chemistry parameters that were significantly different by sex.

Parameter	Sex	Mean	SD	Min–max
Calcium (mg/dL)	Male	12.5[a]	11.8–13.9[b]	11.7–14.4
	Female	>20.0[a]	15.6–20.0[b]	12.0–>20.0
Phosphorus (mg/dL)	Male	4.0	0.9	2.6–6.2
	Female	9.6	4.3	3.8–18.8
Total protein (g/dL)	Male	6.0	0.6	4.9–7.7
	Female	6.6	0.8	5.2–8.0
Albumin (g/dL)	Male	2.7	0.2	2.3–3.2
	Female	2.9	0.3	2.4–3.4
PCV (%)	Male	36.2	4.8	23.0–45.0
	Female	30.6	4.6	24.0–43.0

[a] Median.
[b] 10–90%.

Biochemical analysis—The biochemical analysis for both populations was performed with the use of the avian-reptilian rotor on the first-generation VetScan® analyzer (Abaxis, Inc., Union City, CA). All samples were run with exactly 100 µl of plasma. The avian-reptilian rotor provides analysis of the following parameters: albumin (ALB), aspartate aminotransferase (AST), bile acids (BA), calcium (CA), creatine kinase (CK), globulins (GLOB), glucose (GLU), potassium (K), sodium (Na), phosphorus (PHOS), total protein (TP), and uric acid (UA). The lower end of the dynamic range of the BA on the rotor is <35. Geckos that registered <35 were excluded from the analysis. The higher end of the dynamic range for CA with the rotor was >20. For analysis, a CA >20 was registered as 20.

Statistics—The distribution of the data was evaluated with the use of the Kolmogorov-Smirnov test. Data that were normally distributed were reported by the mean, standard deviation (SD) and minimum–maximum (min–max), whereas nonnormally distributed data were reported by the median, 10–90%, and min–max. A *t*-test or Mann-Whitney test was used to compare each of the hematologic and chemistry parameters by sex for the normally and nonnormally distributed data, respectively. The Mann-Whitney was automatically used for the CA comparison because the data was converted to rank data because of the limits of the higher end of the dynamic range of the rotor. A Spearman's correlation test was used to evaluate certain hematologic and chemistry data for potential associations that could be used to predict health. A $P < 0.05$ was used to determine statistical significance. A power analysis was performed when the alpha was 0.06–0.10 to assess for the potential of a Type-II error. SPSS 18.0 (SPSS Inc., Chicago, IL) was used to analyze the data.

Results

The median weight of the geckos was 43.4 g (10–90%: 38.0–55.3; min–max: 32.0–69.0), and there was no difference in weight between the sexes ($P = 0.2$). The following parameters were found to be significantly different by sex: calcium ($P = 0.0001$), phosphorus ($P = 0.0001$), total protein ($P = 0.03$), albumin ($P = 0.03$), and PCV ($P = 0.001$) (Table 1). The alpha for globulins approached 0.05, but was found to be nonsignificant ($P = 0.06$). A power analysis was performed and found to be 0.5, suggesting the potential for a Type-II error. All of the hematologic and chemistry parameters not found to be different by sex are reported in Tables 2 and 3.

Table 2. Crested gecko hematologic and chemistry parameters that were normally distributed and not significantly different by sex.

Parameter	Mean	SD	Min–max
Glucose (mg/dL)	106.6	33.1	56.0–180
Globulin (g/dL)	3.5	0.6	2.6–5.2
White blood cell ($\times 10^3$/µl)	15.4	7.1	3.5–38.9
Lymphocytes (%)	69.0	11.8	36.0–87.0
Lymphhocytes (absolute) ($\times 10^3$/µl)	10.7	5.1	2.2–24.9

When looking at potential indices that could be used to assess the relationship of minerals and protein we found that there was a strong positive correlation between calcium and phosphorus values (0.74) in crested geckos. The correlation between phosphorus and total protein (0.6) was greater than that found between calcium and total protein (0.47); however, the correlation between the calcium and albumin (0.6) was much stronger than with phosphorus and albumin (0.36). There was a very strong correlation between AST and CK values (0.93) and an inverse weak correlation between AST and bile acids (−0.2). There was a moderately positive association between glucose and PCV (0.5). There was a moderate inverse relationship between total protein and uric acid (−0.43).

On the blood smears, erythrocytes had an oval shape with homogeneous, orange–pink cytoplasm. The RBC nuclei were irregularly round or sometimes misshapen, with dense dark purple, clumped chromatin. In most of the geckos, immature erythrocytes comprised <1% of the erythrocytes present and were slightly smaller than mature RBCs, more rounded, and had slightly basophilic, smooth cytoplasm (polychromasia) (Fig. 2). In the gecko with the lowest PCV (23%), greater numbers of immature erythrocytes were found, including small numbers of rubricytes and occasional prorubricytes (Fig. 3), suggesting that although this gecko appeared to be healthy, there may have been a regenerative response to possible anemia. Early rubricytes and prorubricytes might be mistaken for reactive lymphocytes, but had much more tightly clumped, dark purple nuclear chromatin.

Thrombocyte morphologic features varied considerably, and in some cases were difficult to distinguish from lymphocytes. Many were round to ellipsoidal with a small amount of clear, colorless cytoplasm resulting in a high nuclear to cytoplasmic ratio (N:C ratio). Thrombocytes were characterized by round to oval nuclei with smooth, tightly

Table 3. Crested gecko hematologic and chemistry parameters that were not normally distributed and not significantly different by sex.

Parameter	Median	10–90%	Min–max
A/G ratio	0.8	0.6–1.0	0.5–1.0
Sodium (mmol/L)	143.0	135.9–148.1	134.0–150.0
Potassium (mmol/L)	2.6	1.5–4.5	1.1–6.5
AST (U/L)	30.0	12.0–84.4	9.0–127.0
CK (U/L)	489.0	89.3–2103.8	58.0–3905.0
Uric Acid (mg/dL)	2.6	0.9–6.0	0.8–11.5
Na/K ratio	53.0	31.8–88.8	23.0–134.0
Bile acids[a] (umol/L)	43.0	<35.0–44.0	<35.0–89.0
Heterophils (%)	10.3	5.1–20.9	3.0–39.0
Heterophils (Abs)	1.5	0.6–4.2	0.35–8.4
Monocytes (%)	14.0	7.1–24.9	6.0–33.0
Monocytes (Abs)	1.9	0.8–5.1	0.35–9.02
Eosinophils (%)	0.0	0.0–1.9	0.0–2.0
Eosinophils (Abs)	0.0	0.0–0.2	0.0–0.6
Basophils (%)	2.0	0.0–6.0	0.0–12.0
Basophils (Abs)	0.3	0.0–0.8	0.0–2.0

[a] $N = 5$, all others <35; Abs = Absolute; A/G = Albumin/Globulin; Na/K = Sodium/Potassium; All Abs cell counts = $\times 10^3/\mu l$

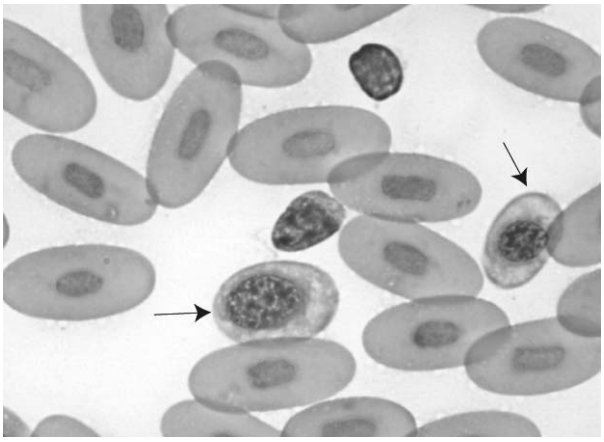

Figure 2. Erythrocytes in a peripheral blood smear from a crested gecko. Note the two immature red blood cells (RBCs) (arrows). Compared to the mature RBCs, these cells are smaller and slightly rounded, with blue-grey cytoplasm (polychromasia). A small lymphocyte, in the center of the field, is much smaller than the immature RBCs, with a scant amount of basophilic cytoplasm concentrated to one side of the nucleus. Aqueous Romanowsky stain, × 1,000 magnification.

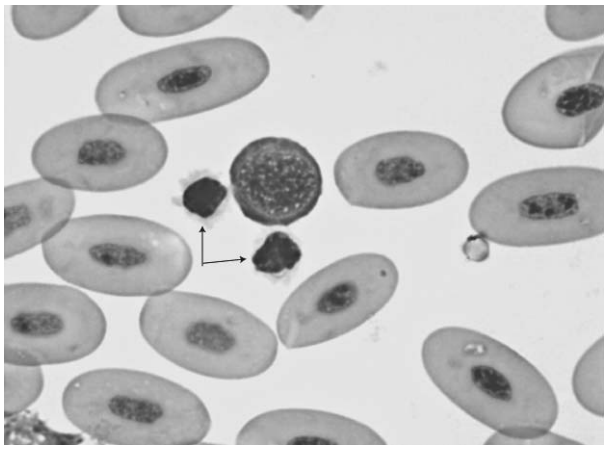

Figure 3. Prorubricyte and two thrombocytes (arrows) in a peripheral blood smear from a crested gecko. This very immature RBC can be distinguished from a reactive lymphocyte by its perfectly round nucleus and tightly clumped chromatin, with a checkerboard-like pattern of light and dark areas. The wispy, irregular cytoplasmic borders suggests that these thrombocytes have been activated and starting to become adherent. Aqueous Romanowsky stain, × 1,000 magnification.

condensed, dark purple chromatin. Often thrombocytes were irregularly round to slightly oval with a small amount of pale, almost colorless cytoplasm with irregular cytoplasmic margins, and a centrally located nucleus (Figs. 3 and 4). Other thrombocytes were extremely small, with a scant amount of wispy, blue–gray cytoplasm with indistinct cell margins that sometimes extended as a tail away from the darkly staining, eccentrically located nucleus. Cytoplasmic vacuoles or granules were not seen. Most samples contained moderate numbers of thrombocytes, with 4–10 thrombocytes/× 100 microscopic field or 50–250 per 100 leukocytes. However, variably sized clusters of thrombocytes were a

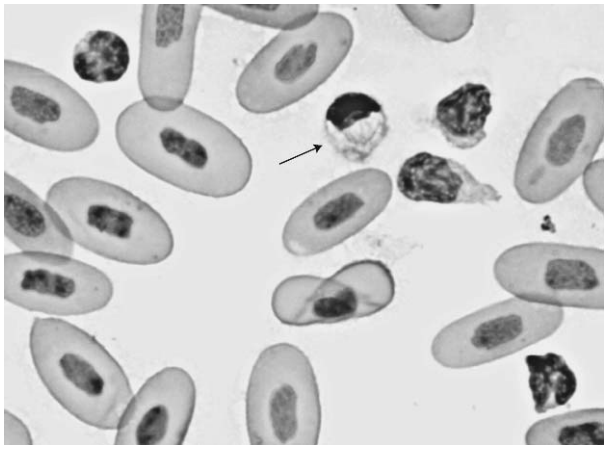

Figure 4. Four lymphocytes and a thrombocyte (arrow) in a peripheral blood smear from a crested gecko. Note that the thrombocyte has almost colorless cytoplasm and tightly condensed, dark purple, nuclear chromatin. Lymphocytes differed by having a small amount of lightly basophilic cytoplasm and pink–purple, slightly clumped nuclear chromatin. Aqueous Romanowsky stain, × 1,000 magnification.

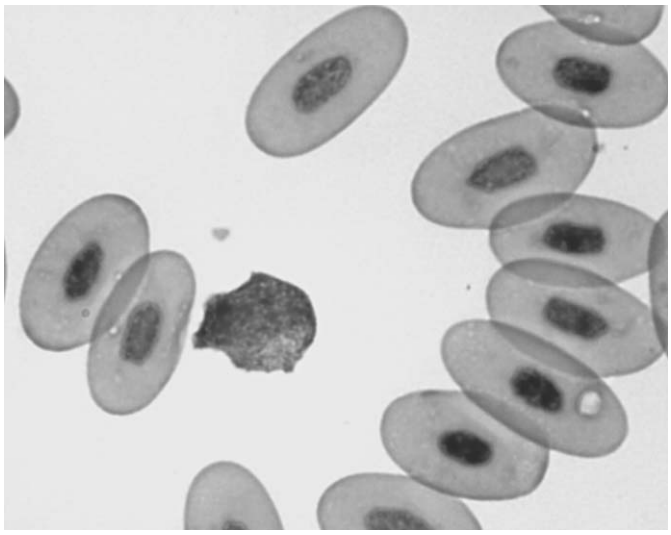

Figure 5. A large reactive-looking lymphocyte in a peripheral blood smear from a crested gecko. Unlike the immature RBCs, this cell has an irregularly round nucleus and relatively loose, light pinkish-purple chromatin. Aqueous Romanowsky stain, × 1,000 magnification.

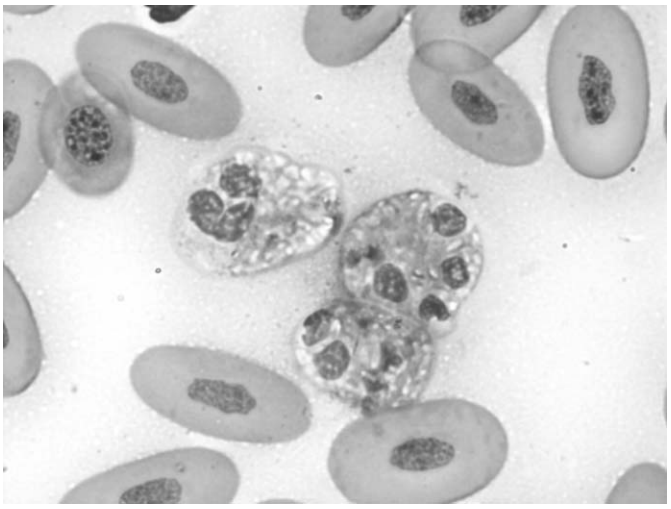

Figure 6. Three heterophils in a peripheral blood smear from a crested gecko. Dull orange, rod-shaped granules fill the cytoplasm that, when visible, is light blue. Nuclei are segmented, but some heterophils appear multinucleated when granules obscure the connections between nuclear lobes. Aqueous Romanowsky stain, × 1,000 magnification.

common finding. The number of thrombocytes in the body of the smear was mildly affected by the tendency of these cells to clump, as well as by their fragility. Lower numbers of thrombocytes were typically found in the smears with larger thrombocyte clumps, suggesting that estimates of the thrombocyte count were inaccurate in these samples. Some smears contained moderate numbers of small free nuclei, presumably derived from broken, smudged thrombocytes.

Lymphocytes were typically the most frequent leukocyte seen on the smears. These cells could be distinguished from thrombocytes by their basophilic cytoplasm and the fact that lymphocyte nuclei had more diffuse, but slightly clumped chromatin that was a lighter pinkish-purple color compared to the dark purple color of thrombocyte nuclei (Fig. 4). Their nuclei were irregularly round or sometimes cleaved. Most lymphocytes were small to medium in size, with diameters of about 6.0–11.0 μm. However, occasional large lymphocytes were also observed, some appearing reactive, with deeply basophilic cytoplasm, and irregularly shaped nuclei with relatively loose, stippled chromatin (Fig. 5).

Heterophil numbers showed significant variation between samples, ranging from 3 to 39% of WBC. These cells contained variable numbers of pink granules that appeared to be oval, elongated, or spindle shaped, and sometimes refractile (Fig. 6). Cytoplasm, when visible, was colorless to pale blue. Heterophils were among the largest leukocytes in the blood, with diameters of 11.0–16.0 μm. Heterophil nuclei were bilobed or multilobulated, with clumped, dark purple chromatin. Because portions of the nuclei were often obscured by granules, nuclear lobes often appeared to be separated from each other, giving the impression of a multinucleated cell.

Monocytes comprised 6–33% of the leukocytes and were variable in size, with diameters ranging from 11.0 to 20 μm. They tended to have a lower nuclear to cytoplasmic ratio (N:C ratio) than lymphocytes due to a moderate amount of granular blue-gray cytoplasm. Many contained variable numbers of dark pink (azurophilic) cytoplasmic granules (azurophils) (Fig. 7) that were sometimes discernible as discrete granules, whereas, in other cells, the granules were extremely small and infrequent and merely imparted a pinkish hue to the cytoplasm. Occasional monocytes were vacuolated or contained phagocytosed debris. A macrophage in the blood smear from one gecko contained some phagocytosed melanin granules (melanophages). Monocyte nuclei varied in shape and were irregularly round, oval or slightly lobulated. Their chromatin had a more lacy appearance than that of lymphocytes.

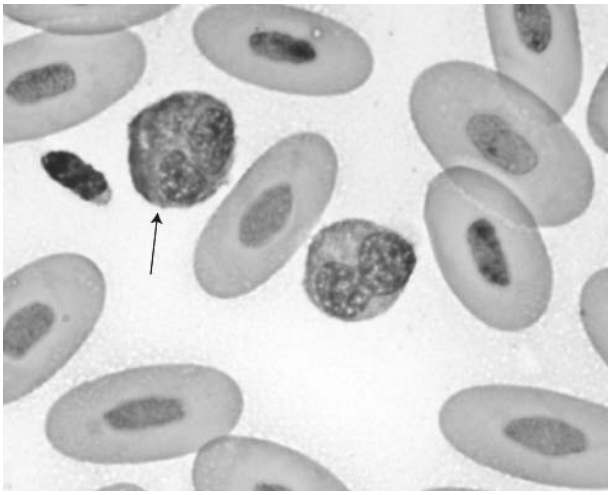

Figure 7. Two monocytes, one (arrow) containing prominent pink cytoplasmic granules (azurophil) in a peripheral blood smear from a crested gecko. Aqueous Romanowsky stain, × 1,000 magnification.

In the examined blood smears eosinophils were uncommon, but could be distinguished from heterophils by their irregularly round, bright orange granules and round, eccentric nuclei (Fig. 8). These cells were similar in size to heterophils, with diameters ranging from 10.0 to 16.0 μm.

Many samples contained a few basophils, which were readily identified by the presence of fine, dark purple (metachromatic) granules. Basophils were similar in size to the small lymphocytes, with diameters of 6.0–11.0 μm. Their nuclei, when visible, were round and often eccentric (Fig. 9). Some of the smears (42%, 16/38) contained a population of cells with numerous sharply defined cytoplasmic vacuoles (Fig. 10). The lack of granules made identification of these cells a challenge. However, these cells were typically small with a round, eccentric nucleus and lightly basophilic cytoplasm, suggesting that these cells were poorly stained or degranulated basophils. No basophils or vacuolated cells were found in 13% (5/38) of the samples.

Discussion

This is the first study to report hematological values for the adult crested gecko. The data presented can be used to provide the veterinary clinician with a specific hematological reference for comparison with crested gecko cases presenting to their practice. This report incorporated one large population of animals that had a standardized environment and diet, were actively breeding, and in which we were able to monitor the long-term effect of the phlebotomy; the animals showed no ill effects from the blood collection for a time span of over 1 yr, giving the study a good foundation for the statistical analysis of the results.

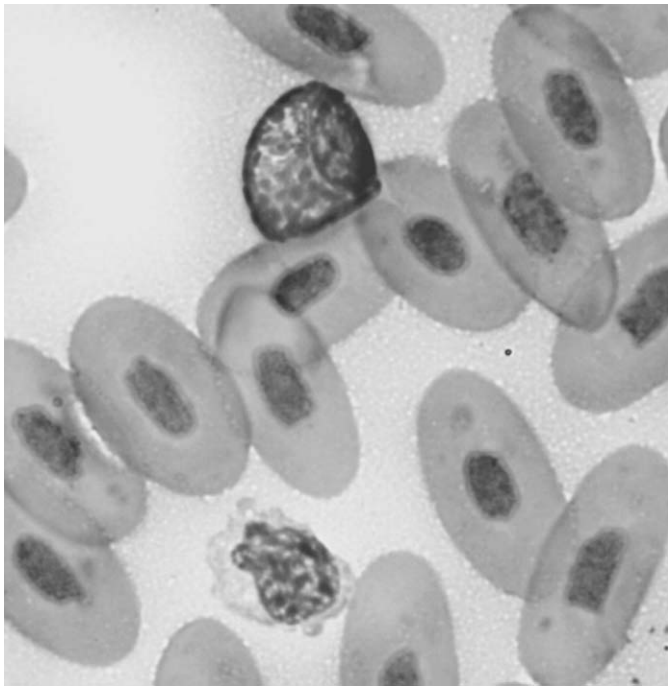

Figure 8. An eosinophil (top) and lymphocyte in a peripheral blood smear from a crested gecko. Eosinophils typically had irregularly round, chunky granules that were a brighter shade of orange than the heterophil granules. Unlike heterophils, which had segmented nuclei, eosinophils had a round nucleus. Aqueous Romanowsky stain, × 1,000 magnification.

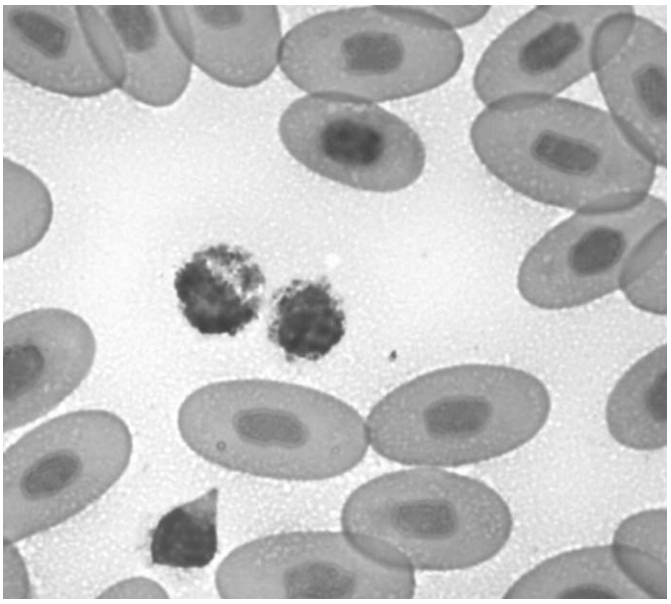

Figure 9. Two basophils (center) and one lymphocyte in a peripheral blood smear from a crested gecko, illustrating the fine, metachromatic granules seen in some basophils. These cells are smaller than other granulocytes, sometimes with a round, often eccentric nucleus. Aqueous Romanowsky stain, × 1,000 magnification.

All females but one from the population had blood calcium values >16.0 mg/dl. The relatively narrow dynamic range for certain values of the analyzer is one disadvantage of the current avian/reptile rotor used in this study. However, values that are slightly higher than the dynamic range

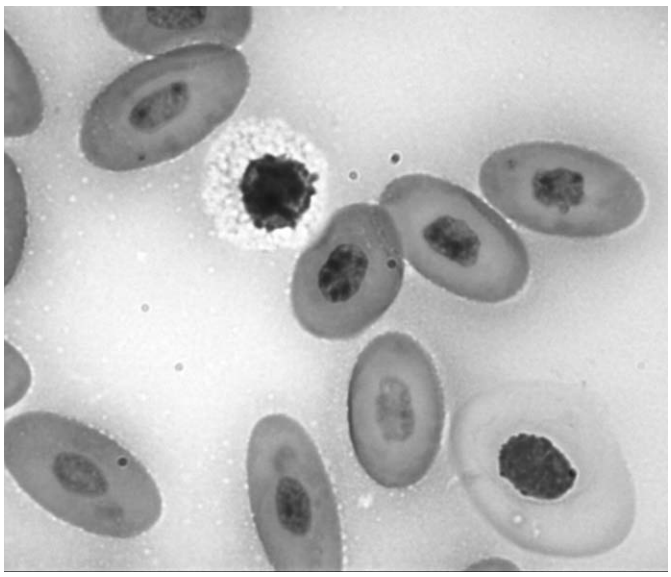

Figure 10. Vacuolated, presumptive basophil in a peripheral blood smear from a crested gecko. The sharply defined granules may reflect some type of staining artifact. Vacuolated cells tended to be smaller than the heterophils and eosinophils. Their nuclei were round, with dark chromatin, similar to the basophils with metachromatic granules. Aqueous Romanowsky stain, × 1,000 magnification.

can still be accessed by the unit after the results are initially suppressed by the printout of the report (a manual override allows one to print the higher values). Values over 20 mg/dl were reported as >20 mg/dl. Each rotor is a single-use plastic rotor. Each reagent rotor contains a diluent and all the profiles necessary to perform a complete multichemistry blood analysis. Usually 100 µl of whole blood is needed in order to obtain all of the electrolyte values from the rotor. Other chemistry analyzers use test strips in a similar fashion. In cases where the true total calcium is required, such as during reproductive evaluations, another analyzer must be used. A similar problem may be encountered when evaluating renal disease in reptiles. Green iguanas with chronic renal failure routinely develop severe hyperphosphatemia (>20 mg/dl) (Mayer, personal observation). Determining an end-point phosphorus level is important when calculating a calcium–phosphorus product to assess the likelihood for metastatic tissue mineralization. The low limit of the rotor regarding total calcium values would not enable the veterinary clinician to make this determination. Some of our females were in active reproductive stages and the significant difference in CA between the males and the females (females had higher values) can explain this sex-based difference.

The literature suggests that sex can affect RBC mass, although there is some conflicting information about this effect. No differences were found between the PCVs in male and female Chinese water dragons (Mayer *et al*., 2005), while Harr *et al*. (2001) found that female iguanas had higher PCVs than males, and Mader (2000) reported that RBC numbers are higher in males than females. The current study found a statistically significant difference between males and females, with males having, on average, PCV that were 15.4% higher than females. Because the crested geckos all originated from a captive environment, it is not known why males would have a higher PCV; it is unlikely that male activity levels, and thus metabolic needs, are any higher than those of female crested geckos in captivity. It is possible that the females have a lower PCV because of blood loss/redistribution during egg development; however, additional study is needed to confirm this. It is also possible that plasma volume may increase during egg production, resulting in a decreased PCV.

The documentation and description of the different white blood cell types will serve as an aid to clinicians and clinical pathologists when evaluating crested gecko blood smears. Heterophils of crested geckos have lobulated nuclei similar to the heterophils of iguanas (Harr *et al.*, 2001) and Chinese water dragons (Mayer *et al*., 2005). This is in contrast to the heterophils of chelonians, crocodilians, snakes, and Gila monsters, which usually have round to oval, nonlobed nuclei (Mateo *et al.*, 1984; Hawkey and Dennett, 1989; Alleman *et al.*, 1992; LeBlanc *et al.,* 2000; Cooper-Bailey *et al.*, 2011). It can be difficult to distinguish heterophils from eosinophils in some reptiles; however, in crested geckos, eosinophils were uncommon and, when present, had larger, darker orange granules as compared to the heterophils.

As reported in other types of reptiles, it was sometimes difficult to distinguish thrombocytes from lymphocytes (McArthur *et al.*, 2010). Larger thrombocytes could be clearly distinguished from lymphocytes by their almost colorless cytoplasm. Smaller thrombocytes could be distinguished from lymphocytes by their scant cytoplasm, with wispy cell margins, and smaller, darker nuclei. Cytoplasmic vacuoles or granules, as often seen in avian (Campbell and Ellis, 2007), iguana (Harr *et al.*, 2001), king cobra (Salakij *et al.,* 2002) and Gila monster (Cooper-Bailey *et al.*, 2011) thrombocytes, were not seen in the crested gecko thrombocytes.

Many of the monocytes contained at least small numbers of fine azurophilic (pink) cytoplasmic granules, and in some cases these granules were numerous. Some consider azurophils, characterized by an oval to bilobed nucleus, basophilic cytoplasm, and small numbers of azurophilic granules, a unique cell type in reptiles (Hawkey and Dennett, 1989; Alleman *et al.*, 1992). However, cytochemical staining of these cells suggests that they are a type of monocyte, perhaps an immature form, and that there is little clinical advantage in separating azurophils from monocytes in the leukocyte differential count (Campbell and Ellis, 2007). In this study these cells were counted as monocytes.

Some gecko blood smears had basophils with typically dark purple granules. Largely because of their small size, but also because of the round, eccentric nucleus, vacuolated cells were also interpreted to be basophils. The poor staining of basophil granules can likely be attributed to the use of an aqueous Romanowsky stain. Romanowsky stains, which combine a basic thiazine dye (e.g., azure A or methylene blue) and an acidic dye (e.g., eosin Y or eosin B), exist as either aqueous or methanolic solutions. Dip stains such as Diff-Quik and Hema 3 are water-based Romanowsky stains, with an initial methanol fixation step. In contrast, many of the automated modified Wright stains are methanolic stains. The automatic slide stainer used in this study utilized an aqueous Romanowsky stain. Hawkey and Dennett (1989) reported that degranulation of heterophils and basophils was a possible side effect of inadequate sample fixation, presumably allowing loss of aqueous granule content during processing. It has been shown that the cytoplasmic granules of mast cells, basophils, and large granular lymphocytes stain poorly or not at all with aqueous Romanowsky stains (Rebar *et al.*, 1982; Leclere *et al.,* 2006; Allison and Velguth, 2010). In fact, Allison and Velguth (2010) demonstrated that with an aqueous Romanowsky stain, nonstaining ostrich basophils appear to contain large vacuoles similar to the cells found in this study, whereas the same cells contain dark purple granules when the blood smear is stained with a methanolic stain. Aqueous-based quick stains such as Diff-Quick may also fail to stain eosinophils adequately in some dog breeds, especially greyhounds (Iazbik and Couto, 2005). However, the vacuolated cells in this study were not as large as the recognizable eosinophils. It is possible that the basophil results reported in this study are biased because of the stain type used for the blood smears.

Veterinarians working with reptiles frequently find themselves with limited comparative information for interpreting diagnostic test results, and although crested geckos have gained in popularity in recent years, there has been limited research investigating diagnostic testing related to this reptile. Because hematology and clinical biochemistries provide invaluable insight into the health and physiologic condition of a patient, the authors felt a study to provide some reference material for veterinarians working with these animals would be helpful. The authors realize that the presented data have been established from a single colony and care has to be taken not to overinterpret the results as a reference range; instead these data are intended as a guideline for general comparison.

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